DNA YFILER INTERPRETATION GUIDELINES

A. SCOPE

A.1 The interpretation of results in casework is necessarily a matter of professional judgment and expertise. Not every situation can or should be covered by a pre-set rule. However, it is important that the laboratory develops and adheres to minimum criteria for interpretation of analytical results. These criteria are based on validation studies, literature references, and casework. It is to be expected that these interpretation guidelines will continue to evolve as the technology and collective experience of the laboratory grows.

A.2 Y STR data might be used in conjunction with the autosomal STR results and both methods can assist the interpretation of each other. In some circumstances Y STR data might be the only data that can be obtained. It is important to note that a Y STR haplotype is shared by males from the same paternal lineage, which should be taken into account when drawing conclusions.

A.3 The purpose of these guidelines is to establish a general framework and outline minimum standards to ensure that:

A.3.1 Conclusions in casework reports are scientifically supported by the analytical data, including that obtained from appropriate standards and controls.

A.3.2 Interpretations are made as objectively as possible, consistently from analyst to analyst, and within established limits.

A.4 The goal of the evaluation and interpretation of amplified Y STR data is to determine the DNA profile(s) of the questioned samples for comparison to male reference sample profiles.

A.4.1 A peak is defined as a distinct, triangular section of an electropherogram.

A.4.2 Genotypes are determined from the diagnostic peaks of the appropriate color and size range for a particular locus. The combination of the genotypes is deemed the Y haplotype.

B. PRELIMINARY EVALUATION OF YFILER DATA

B.1 *ANALTYICAL THRESHOLD AND OPTIMAL PEAK HEIGHT*

The analytical threshold for YFiler is 50 relative fluorescent units (RFU). Optimal peak height values range between 50-6000 RFU, although acceptable and typeable signals may occur outside of this range.

B.2 *LADDER*

The ladder used for sizing must be evaluated to determine that all peaks type correctly, no spikes exist in the sizing range, and the peak heights are above 50 RFU. This evaluation may be performed by GeneMapper ID-X.

B.3 *INTERNAL SIZE STANDARD*

The internal size standard utilized is GeneScan 500 LIZ. The internal size standard must be evaluated to determine that the expected pattern is present; this evaluation may be performed by GeneMapper ID-X. The 250 peak is not designated by the software. The LIZ run with samples should be within +/- 0.5 bases of the 250 peak in LIZ run with the ladders utilized for allele designation.

B.4 *INTERPRETATION OF CONTROL SAMPLES*

B.4.1 *REAGENT CONTROLS*

A reagent control tests for the possible presence of contamination of the extraction reagents and/or supplies by an adventitious source of DNA. The adventitious DNA can be non-amplified DNA or PCR product. If only a single peak (not attributable to a spike or other artifact) exists in the reagent control electropherogram between 75 and 450 bp and there are no signs of any other peaks, this will not be reported as contamination unless this sample has a quantitation value of 10^{-3} ng/µL or higher. Conversely, if there is a single peak in the reagent control electropherogram (not attributable to a spike or other artifact) but signs of other peak(s) are visible, this will be reported as contamination, even if this reagent control did not have a quantitation value. However, due to the low level of DNA present and the inability to draw any conclusions for this single result, this contamination will typically not affect any conclusions drawn for corresponding questioned samples. If, between 75 and 450 bp, a reagent blank exhibits multiple peaks at or above 50 RFU not attributable to spikes or other artifacts, the questioned samples extracted with the reagents contained in the reagent blank will be considered inconclusive for match purposes unless it can be determined that the contamination is isolated to the reagent control and did not affect the evidentiary samples. This can be accomplished through additional samples extracted simultaneously that exhibit no peaks, e.g. a second reagent control, water control, or a sample containing no DNA. Where possible, the analyst may go back to the earliest item of evidence associated with the blank e.g. raw evidence material, to try to reproduce or eliminate the contamination. Efforts should be made to determine the source of contamination. If contamination cannot be deemed isolated to just the reagent blank and reanalysis is not possible then these questioned samples cannot be used for

inclusions or exclusions. Reagent blanks containing spikes between 75 and 450 bp may be re-injected. Spikes or other artifacts that fall outside these areas are not significant. The Technical Leader must be informed of any contamination event and the contamination log must be filled out. Contamination events will be documented in the case package and report.

B.4.2 *NEGATIVE CONTROL*

A negative control (amplification blank and/or female negative control) tests for the possible presence of contamination occurring during amplification set-up or female cross reactivity. If, between 75 and 450 bp, a negative control exhibits any peaks (≥50 RFU) not attributable to a spike or other artifact, the DNA specimens amplified at the same time as the negative control will be considered inconclusive for match purposes unless it can be determined that the contamination is isolated to the negative control and did not affect the evidentiary samples. This can be accomplished through additional samples extracted simultaneously exhibiting no peaks, e.g. a reagent control, water control, or a sample containing no DNA. Where possible, the analyst may go back and re-amplify all samples associated with this amplification negative control. If contamination cannot be deemed isolated to just the negative control and re-analysis is not possible, then no conclusions will be offered for these samples. Negative controls containing spikes or other artifacts, between 75 and 450 bp, may be re-injected. Spikes or other artifacts that fall outside these areas are not significant. The Technical Leader must be informed of the contamination event and contamination of the negative control will be documented in the contamination log, case package, and where appropriate in the report.

B.4.3 *POSITIVE CONTROL*

Specimen 007 is a positive control for the Y-filer STR amplification system. This positive control is used to evaluate the performance of the amplification and typing procedure.

DYS456	DYS389 1	DYS39 0	DYS3891 1	DYS458	DYS19	DYS38 5	DYS393	DYS391	DYS439
15	13	24	29	17	15	11,14	13	11	12

DYS635	DYS392	GATA	DYS437	DYS438	DYS448
24	13	13	15	12	19

If the 007 control does not exhibit the Y STR typing results listed above, the following steps must be taken.

B.4.3.1 If there appears to be an injection or electrophoretic problem, re-inject the 007 control. Alternatively, the amplified product may be re-set up for 3130 electrophoresis.

B.4.3.2 If re-injection of the 007 control does not resolve the problem and there may be amplification issues, all samples co-amplified with this control will be considered inconclusive for matching purposes at each locus where the 007 control did not exhibit results at or above 50 RFU unless a QC sample (an in-house sample with a known profile) was amplified concurrently and that sample gave the expected profile with all results at or above 50 RFU. If the issue is a low level positive control, the results from co-amplified samples can still be used for purposes of exclusion. If sufficient DNA remains from samples co-amplified with a failed control and no QC sample was co-amplified, then re-amplification is appropriate. If the 007 control yields accurate typing results when re-amplified, then the samples amplified with this control will be considered acceptable for matching purposes.

B.4.3.3 If results of the 007 show an incorrect allele call, all samples co-amplified with this control will be considered inconclusive.

B.5 *DESIGNATION*

B.5.1 *LOCUS AND ALLELE DESIGNATION*

The GeneMapper ID-X program will be used for the locus and allele designations of the raw data. The examiner must review the GeneMapper ID-X calls and create a table of results from this review.

B.5.2 *OFF LADDER VARIANTS*

Off ladder alleles that fall between alleles within the ladder will be designated in accordance with guidelines of the International Society for Forensic Haemogenetics. Off ladder (OL) calls are first converted to size in base pairs (bp), and then compared to the size of the appropriate ladder alleles and the allelic designation determined. If the OL is not a "perfect" repeat, but rather varies by 1, 2 or 3 bp from a ladder allele, then it will be designated as an integer of that variation. For example, if an OL peak size is 238.39 bp, and the 15 allele of the ladder is 236.32 bp, then the peak will be designated as 15.2. If an allele falls

above the largest or below the smallest peak of the sizing ladder, the allele will be designated as either greater than (>) or less than (<) the respective ladder allele.

Non-artifactual off-ladder alleles will generally conform to the same overall guidelines described above. An analyst must re-inject (or re-amplify) any off ladder alleles in a sample for verification unless the results from that sample will not be interpreted.

B.5.3 *ARTIFACTS*

Artifacts can occur and need to be recognized. These may include, but are not limited to, the following: spikes, pull-up, stutter, and non-template nucleotide addition.

B.5.3.1 *SPIKES*

Peaks of the same size and similar height (within an order of magnitude of each other) in all four or five colors are not the result of dye-labeled DNA and do not represent a spectral problem. These are artifacts due to the presence of fluorescent material in either the formamide or the POP-4 polymer. These peaks can be shown to be artifacts by re-injection of the sample.

B.5.3.2 *STUTTER*

In addition to an allele's primary peak, artifactual minor "stutter" peaks can occur at three and four-base intervals. The most common stutter peaks observed in all loci are three or four bases smaller than the primary peak (n-4) in four base repeats and three bases smaller (n-3) in three base STR's. It is also possible to see additional "n+3 and n+4" peaks (three or four bases larger), especially when excessive amounts of DNA are amplified. See the below table for stutter types. Stutter peaks are evaluated by examining the ratio of the stutter peak height to the height of the appropriate adjacent allele, expressed as a percentage. The height of stutter peaks can vary by locus, and longer alleles within a locus generally have a higher percentage of stutter. The percentage stutter expected for each locus as seen in the table below is utilized in Genemapper ID-X. These are the highest expected stutter percentages for each locus based on the manufacturer's developmental validation.

Locus	Stutter Type (bp)	Stutter Ratio (%)
DYS456	**N-4**	**13%**
DYS3891	**N-4**	**12%**
DYS390	**N-4**	**10%**

DYS389II	**N-4**	**14%**
DYS458	**N-4**	**12%**
DYS19	**N-4**	**11%**
DYS19	**N-2**	**10%**
DYS385	**N-4**	**14%**
DYS393	**N-4**	**13%**
DYS391	**N-4**	**12%**
DYS439	**N-4**	**11%**
DYS635	**N-4**	**11%**
DYS392	**N-4**	**16%**
DYS392	**N+3**	**8%**
GATA_H4	**N-4**	**11%**
DYS437	**N-4**	**9%**
DYS438	**N-5**	**4%**
DYS448	**N-6**	**5%**

Peaks in the stutter positions greater than these values may indicate the presence of a mixture. In addition to a mixed sample, stutter peaks may be elevated above established thresholds by the following:

Analyzed peak heights above the optimal range (approximately 50-6000 RFU) may be "off-scale" in the raw data, meaning that the CCD camera may be saturated. If any raw data peaks have a height of 8191 RFU, they are off-scale. While the GeneMapper ID-X software will alert the analyst to any off-scale raw data peaks, the analyzed peak may be assigned a lower value due to smoothing and base-lining functions. Therefore, the observed percent stutter will be inaccurately high. If the stutter peak is greater than the maximum allowed and the primary peak is above 6000 RFU and/or has been labeled off-scale, the analyst should interpret the results with caution. The sample may be re-amplified with less input DNA or re-inject for a shorter period of time.

Stutter peaks overlapping an area of elevated baseline may exhibit increased percentages. Elevated baselines may indicate an improperly functioning spectral.

B.5.3.3 *NON-TEMPLATE NUCLEOTIDE ADDITION (–A)*

Amplification conditions have been set to maximize the non-template addition of a 3´ terminal nucleotide by AmpliTaq Gold DNA polymerase. Failure to attain complete terminal nucleotide addition results in "peak splitting", visualized as two peaks one base apart. This is most often seen when an excessive amount of DNA is amplified or amplification is performed under sub-optimal PCR conditions. Excepting micro-variants, the presence of peaks differing by one base pair is diagnostic of this problem.

B.5.3.4 *OTHER ANOMALIES*

In addition to amplification artifacts described above the following anomalies can arise during electrophoresis and analysis:

Significant room temperature fluctuation may result in electrophoretic variations between injections such that allelic ladder peaks differ by more than 0.5 bp from allelic peaks in other injections. This will disrupt sample analysis using the GeneMapper ID-X software. Analyzing samples with an injection of allelic ladder nearest the questioned samples may alleviate this problem. If desired, the sample(s) and an allelic ladder may be re-injected to confirm the typing.

Small artifactual peaks can appear in other colors under true peaks. This phenomenon is termed "pull-up". Pull-up is a result of spectral overlap between the dyes, which is normally corrected for by the spectral. If a pull-up peak is above the minimum peak height detection threshold, it will be sized at the same size as the true peak. Pull-up can occur as a result of the amplification of excess input DNA resulting in off-scale peaks (see above). The spectral may not perform properly with off-scale data.

Artifactual peaks of a single color will not display the typical spectral overlap characteristic of the four fluorescent dyes in the raw data. Peak width may not be similar to the peaks resulting from dye-labeled DNA. These peaks can be shown to be artifactual by re-injection of the sample.

B.6 *INTERPRETATION OF RESULTS*

B.6.1 *DETECTION AND INTERPRETATION OF SINGLE SOURCE SAMPLES*

In general, a sample is consistent with being from a single source if each locus typed has only one allele (or two alleles at DYS385). For an apparent single-source questioned sample, compare the results obtained from the questioned sample with the results from the known samples in the case. The determination of inclusions or exclusions is the responsibility of the analyst working the case. This determination is based on all tests and observations made for that sample. In general, if any Y-STR locus doesn't match between a single source questioned sample and a reference sample, then an exclusion can be made regardless of how many other loci match. An exception to this can occur with partial DNA profiles due to DNA degradation, inhibition of amplification, and/or low template quantity and in paternity testing because of the possibility of mutational events.

Statistical interpretation may be applied at loci showing peaks at or above 50 RFU. The frequency of the haplotype will be calculated with the application of a 95% confidence interval utilizing the U.S. Y-STR Database (See DOC ID 1777).

B.6.2 *DETECTION OF MIXTURES*

Samples may contain DNA from more than one individual. A sample is consistent with being a mixture if it exhibits one or more of the following characteristics at more than one locus.

B.6.2.1 More than one allele is present at a locus other than DYS385 after stutter and other artifact considerations have been evaluated and dismissed as possible causes.

B.6.2.2 A peak is present at a stutter location and its height is greater than the highest expect stutter ratio for the locus.

B.6.3 *INTERPRETATION OF MIXED SAMPLES*

The interpretation applied to a mixed sample by the analyst in each particular case should be based upon all relevant information.

B.6.3.1 If there is a dominant male profile present in the mixture, one can determine any inclusions or exclusions by comparing the profiles from known reference samples with the dominant DNA haplotype. Dominant types of a mixture can be assigned based on peak heights as well as peak height ratios. The estimated frequency of the haplotype will be calculated with the application of a 95% confidence interval utilizing the U.S. Y-STR Database (See DOC ID 1777).

B.6.3.2 Information can be gained from minor profiles observed in mixtures as to possible inclusion or exclusions of sources. If a single source minor haplotype can be extracted at any particular locus then the estimated frequency of the haplotype will be calculated with the application of a 95% confidence interval utilizing the U.S. Y-STR Database (See DOC ID 1777).

B.6.3.3 When an indistinguishable mixture exists, i.e. a mixture that does not have a distinct contrast in signal intensities among different contributors, inclusions will not be made as statistical applications for this type of Y-STR mixture have not been evaluated. However, exclusions can be made if the known Y-STR profile contains an

allele or alleles not present in the mixture profile and there is no reason to believe allele dropout has occurred.

B.6.3.4 In some cases one of the male contributors (e.g. the victim) is known and the genetic profile of the unknown may be readily inferred. This can be accomplished by subtracting the DNA contribution of the intimate sample from the mixed profile resulting in a deduced foreign DNA profile.

B.6.4 ***INCOMPLETE STR PROFILES***

The possibility exists that not every locus will amplify. This can occur if the DNA is of limited quantity, degraded or if the DNA sample contains PCR inhibitors. Y-STR results do not have to be obtained from a minimum number of loci for interpretation. Exclusions and inclusions, along with statistical applications, can be determined using any loci that give results at or above 50 RFU.

B.7 ***CONCLUSIONS***

B.7.1 The questioned sample and the known sample contain the same haplotype: Conclusions can be made that the source of the known sample or any of his male biological paternal relatives are consistent with being the source of the questioned sample. The matching haplotype shall be searched in the U.S. Y-STR Database. The estimated frequency of this haplotype will be calculated by utilizing the consolidated data for all population groups that comprise the U.S. Y-STR Database with the application of a 95% confidence interval (See DOC ID 1777).

B.7.2 The questioned sample and the known sample do not contain the same genotype: The source of the known sample and his male biological paternal relatives are excluded as being the source of the questioned sample when the obtained profiles differ at least one locus.

Note: The possibility of a mutation(s) should be considered whenever a biological relationship is in question between two males and there is a difference at one or more Y-STR loci.

B.7.3 When an overall low-level profile is obtained, results may be deemed inconclusive or uninterpretable.

B.7.4 A sample may be reported out as no results obtained when no signal at or above 50 RFU is detected.

B.7.5 **Mixture notations**: when interpreting data from mixture DNA results, clear notes must be made as to the assumptions and reasoning behind the interpretation(s) and conclusion(s) reached. Ordinarily these will be handwritten on the associated electropherogram.

B.8 REFERENCES

B.8.1 Forensic DNA Typing, John M. Butler, Academic Press 2001 and 2005.

B.8.2 WCSO validation of Yfiler.

B.8.3 Y-chromosome Short Tandem Repeat (Y-STR) Interpretation Guidelines Scientific Working Group on DNA Analysis Methods (SWGDAM), Forensic Science Communications, January 2009 - Volume 11 - Number 1.